Avalon Medical™
Innovative Veterinary Surgical Products

PoliPhase

ISO Muscle Implantation Study – 3 Week

Test Article: BIOCOMPOSITE (PoliPhase)

Identification Number: L21-7

Test Facility:
NAMSA
2261 Tracy Road
Northwood, OH 43619-1397

Sponsor
Kem Schankereli
124 S. Second Street #110
Stillwater, MN 55082

TABLE OF CONTENTS

SUMMARY

The test article, BIOCOMPOSITE, Code: L21-7, was implanted in muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity based upon the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 6: Tests for Local Effects after Implantation.

Implant samples and negative control samples were sterilized by ethylene oxide and then degassed for 5 days. Rabbits were implanted and were then euthanized 3 weeks later. Muscle tissue was excised and the implant sites were examined to further define any tissue response.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative control implant material. Microscopically, the test article was classified as nonirritant as compared to the negative control article.

Study and Supervisory Personnel:

- Meridith A. Baugher, RVT, ALAT
- Christine Szych, RVT, ALAT
- Linda L. Tower
- Rachel K. Schroeder, BS
- Julie Horn, RVT, LAT
- Fawn Jones, HT-AD
- Laura Westergaard-Ottinger, BS
- Sara S. Hartman, ALAT
- Amy S. Hershberger, RVT, ALAT
- Brian Brooks
- Mark J. Menzel, RVT
- Deedee M. Davis, BA
- Martha Oswanski, BS, LAT

Approved by: Joshua D. Moninger, BS, Technical Reviewer
Date Completed: July 18, 2003

INTRODUCTION

The test article identified below was evaluated for biocompatibility based upon the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 6: Tests for Local Effects after Implantation. The purpose of the study was to evaluate the potential for a local irritant or toxic response to material implanted in direct contact with muscle tissue. The test article was received on May 16, 2003. The animals were implanted on June 2, 2003, and muscles were explanted on June 23, 2003.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	BIOCOMPOSITE (PoliPhase)
Identification #:	Code: L21-7
Storage Conditions:	Room Temperature
Negative Control Article:	USP polyethylene negative reference strips were purchased by NAMSA from the offices of the US Pharmacopeial Convention and were cut into approximate 1 mm x 10 mm sections.
Preparation:	A minimum of four sections of the test article per rabbit, each Approximately 1 mm x 10 mm were loaded into 16 gauge needles. For each rabbit, a minimum of four negative control samples were loaded into the same size needles as used for the test article. Test and control articles were sterilized by ethylene oxide gas (EO) and then degassed for 5 days prior to implantation.

METHODS

Test System:

Species:	Rabbit (Oryctolagus cuniculus)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	Male
Body Weight Range:	3.5 kg to 4.1 kg at implantation
Age:	No particular age is prescribed for this test
Acclimation Period:	Minimum 5 days
Number of Animals	Three
Identification Method:	Ear Tag

Justification of Test System:

The rabbit is suggested as an appropriate animal model for evaluating polymer materials by the current ANSI/AAMI/ISO testing standards. The muscle tissue has been used historically because the response to implanted material is easily graded and compared to known negative control material.

Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based upon the "Guide for Care and Use of Laboratory Animals."
Food:	PROLAB High Fiber Rabbit Diet was provided daily.
Water:	Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
Housing:	Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex and date implanted.
Environmental:	The room temperature was monitored daily. The temperature range for the room was within a range of 61-75 degrees F. The humidity range for the room was 30-70%. The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
Facility:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institute of Health, Office for Laboratory Animal Welfare.
Personnel:	Associates involved were appropriately qualified and trained.
Selection:	Healthy animals were selected. To reduce the number of animals used for testing, and to comply with the directives of the NAMSA IACUC, rabbits on this study were used previously in an unrelated test model. Any previously evaluated test or control articles did not cause a response to the animals. Complete history of animal usage is traceable in laboratory records. Animals used for previous evaluations are identified in the report.

Experimental Procedure:

Rabbits were clipped free of fur over the paravertebral muscles. An intramuscular injection of a combination of ketamine hydrochloride and xylazine general anesthetic was administered to each animal at a dose of 0.6 ml/kg. Each rabbit was then injected subcutaneously with 0.02 mg/kg buprenophine. After the anesthetic had taken effect, the surgical site was scrubbed with a germicidal soap, wiped with 70% isopropyl alcohol, painted with povidone iodine.

One incision was made on each side of the back through the skin and parallel to the lumbar region of the vertebral column. A stylet was placed in the hub of a loaded needle. The skin was moved to the desired location and the needle was inserted through the incision into the muscle at a 45 degree angle. The needle was withdrawn over the stylet, leaving the sample in the paravertebral muscle. Four test article

sections were implanted in the right paravertebral muscle of each rabbit. Test article sections were placed at appropriately spaced intervals. In the opposite muscle, four negative control sections were similarly implanted. The skin incisions were closed with tissue glue. Rabbits were returned to their respective cages following the procedure and monitored for recovery from the anesthetic. Rabbits were observed daily for general health. Body weights were recorded prior to implantation and at termination.

At 3 weeks, the rabbits were euthanized by an intravenous injection of a sodium pentobarbital based drug. The paravertebral muscles were dissected free and fixed in 10% neutral buffered formalin (NBF) to facilitate cutting. After fixation, the muscles were methodically cut to locate test and control sites. All test and control sites were accounted for. Capsule formation or other signs of irritation were scored using low magnification and the scores were recorded as follow:

0 - No capsule, no adverse reaction (other than minimal hemorrhage)
1 - Up to 0.5 mm capsule or reaction area
2 - 0.6 to 1.0 mm capsule or reaction area
3 - 1.1 to 2.0 mm capsule or reaction area
4 - >2.0 mm capsule or reaction area

Representative tissue implant sites (test and control) from each rabbit were excised, allowing a sufficient area around the site for proper histological preparation. These sections were histologically processed (embedded, sectioned and stained in hematoxylin and eosin) for microscopic evaluation.

Evaluations and Statistics:

The average macroscopic score for test implants was compared with the average score of the control sites. Calculations were rounded off to the nearest 0.1. A Reaction Index difference of 0.0 to 0.5 in scores (test minus control) was regarded as “not significant,” 0.6 to 1.0 as a “trace,” 1.1 to 2.0 as “slight,” 2.1 to 3.0 as “moderate” and ≥ 3.1 as “marked.”

A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response. The evaluation was conducted by a qualified pathologist. The microscopic irritant response was graded as nonirritant, slight, moderate or severe.

RESULTS

Clinical Observations: All animals appeared clinically normal throughout the duration of the study. Body weight data for individual rabbits were considered acceptable.

Macroscopic Observations: There was no visible reaction at any test or control site. This resulted in a macroscopic reaction of “not significant” tissue contact irritation. The findings for the macroscopic evaluation are shown in Appendix 1.

Microscopic Observations: The test article was a nonirritant as microscopically compared to the negative control implant material. Individual results of the pathology findings appear in the microscopic evaluation report.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of the data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and ISO 17025.

CONCLUSION

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative control implant study. Microscopically, the test article was classified as a nonirritant as compared to the negative control article.

RECORD STORAGE

All raw data, paraffin blocks and tissue slides pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

APPENDIX 1

BODY WEIGHTS AND MACROSCOPIC OBSERVATIONS

Rabbit Number/ Gender	Weight (kg) Day 0	Weight (kg) Day 21	Test	Negative Control
33384* Male	3.5	3.7	0 0 0 0	0 0 0 0
33497* Male	3.7	4.0	0 0 0 0	0 0 0 0
33526* Male	4.1	4.2	0 0 0 0	0 0 0 0

* Previous use history traceable in laboratory records.

APPENDIX 2

ISO MUSCLE IMPLANTATION STUDY
MICROSCOPIC EVALUATION

Test Article: BIOCOMPOSITE
Interval Implanted: 3 Weeks

	TEST			HDPE- NEGATIVE CONTROL PLASTIC		
Rabbit #	33384	33497	33526	33384	33497	33526
Inflammation Polymorphonuclear	1	1	1	1	1	1
Lymphocytes	1	1	1	1	1	
Plasma Cells	0	0	0	0	0	0
Macrophages	1	1	1	1	1	1
Giant Cells	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0
SUB TOTAL (X2)	6	6	6	6	6	6
Fibroplasia	0	0	0	0	0	0
Fibrosis	1	1	1	1	1	1
Fatty Infiltrate	0	0	0	0	0	0
SUB TOTAL	1	1	1	1	1	1
TOTAL	7	7	7	7	7	7
GROUP TOTAL	21			21		
AVERAGE* TEST 7.0 (-) CONTROL 7.0 = 0.0 *Used to determine Irritant Ranking Score shown below as the Conclusion. A negative difference was recorded as zero.						
Traumatic Necrosis	0	0	0	0	0	0
Foreign Debris	0	0	0	0	0	0
# Sites Examined	4	4	3	4	4	4

Conclusion:

Under the conditions of this study, the test article was considered a
_X_ Nonirritant (0.0-2.9), ___ Slight Irritant (3.0-8.9), ___ Moderate Irritant (9.0-15.0), ___ Severe Irritant (≥15.1) to the tissue as compared to the HDPE Negative Control Plastic.

Comments: One test site for animal number 33526 was associated with moderate surgical trauma. This new site was not included in the analysis.

Pathologist: T. Muench DVM, MS, PhD Date: 7-18-07

Tim Muench, DVM, MS, PhD
Research Pathologist